PREPARATION, CHARACTERIZATION AND PHRAMACOLOGICAL ASSESSMENT OF NANOSTRUCTURAL FENUGREEK OIL CONTAINING HYPOGLYCEMIC AGENT (ANTI DIABETIC)

A Synopsis
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CONTENTS

1. INTRODUCTION
2. PLANTPROFILE
3. AIM AND OBJECTIVES
4. REVIEW OF LITERATURE
5. METHODOLOGY
6. REFERENCES

INTRODUCTION:

Diabetes is defined as the disease which occurs when the pancreas does not produce enough insulin or in case when the body does not properly uses the insulin. It is a chronic metabolic disease. The symptoms of diabetes are dehydration, frequent urination, nausea, vomiting, fatigue, frequent weight gain or frequent weight loss, gum disease, cut or wound don't heal properly, sexual dysfunction. Diabetes leads to diabetic retinopathy, renal failure, diarrhea cardiovascular, foot ulcers, peripheral vascular, high blood pressure, nerve damage, nausea, constipation, risk of cholesterol, failure of organs. (Samreen et. al.,2009). Several processes are involved in the diabetes development. It involves the beta cells destruction of the pancreas with deficiency of insulin. Due to the deficient action of the insulin, the abnormalities in the carbohydrate, fat and protein metabolism occurs on the targeted tissues due to insensitivity or insulin deficiency.

Types

a) Type 1 diabetes: identified by the lack of insulin production, mainly due to the autoimmune beta cell destruction. In the case of diabetes type 1, the patient has to take daily administration of insulin otherwise it will be fatal. Mainly autoimmune, genetic and environmental factors are involved.

b) Type 2 diabetes: is due to the loss of beta cells insulin cells, results in the unsuccessful use of insulin. It occurs mainly due to the age, obesity, genetic factors, and gestational diabetes.

c) Gestational Diabetes: mainly occurs in pregnancy's second and third semester. Sometimes the gestational pregnancy improves after delivery. Chances of Central nervous system and skeletal system abnormality, high birth weight.

d) The other different types of diabetes are: monogenic diabetes syndromes and maturity onset diabetes of the young, exocrine pancreas disease and drug or chemical induced diabetes (ADA et. al., 2007).

Complications of Diabetes:

Diabetes may lead to nausea, vomiting, deep breathing, and diarrhea. The major complications of the disease are cardiovascular disease, damage to the blood vessels, peripheral vascular diseases, retinopathy (damage to the blood vessels in the retina), foot ulcers, nephropathy (damage to the kidney) leads to chronic kidney diseases like urine protein loss kidney damage. The other complications may be muscle wasting, weakness.

Diagnosis of Diabetes: (Samreen et. al.,2009).

1. Assessment of family history, genetic factors, obesity, age
2. Digiscope

3. Fasting lipid profile
4. TSH and LFT
5. Urine micro albumin measurement
6. Serum creatinine
7. Diagnosis of Blood glucose level
8. Foot exam
9. A1C

Nanoparticles:

Novel drug delivery system increases the patient compliance and bioavailability and repeated administration, It increases the therapeutic value, reduces the toxicity and increases the toxicity. Nanoparticles carry the drug to their specific site by bypassing the all barriers for example the liver metabolism, acidic pH of the stomach. The nanoparticles remain in the blood for a long time due to their small size. The use of herbal medicines with the NDDS improves the various chronological diseases. There are several advantages of herbal drugs in the nanoparticles for example solubility enhancement, increased bioavailability, stability enhancement, enhanced pharmacological activity, sustained drug delivery, proper distribution of drug, toxicity protection and physical and chemical degradation protection. Among the novel drug delivery systems, nanoparticles are considered to be an important one. Because the herbal medicines in the nanoparticles are used to target the disease which improves the drug delivery, safety, therapeutic and pharmacological activity, targeted site specific of the drug, improves the patient compliance by reducing the dose concentration. The nanoparticles should be small enough so that it can reach the target cells and tissues by circulating in the blood stream. Herbal medicines can be targeted to various organs such as brain, lung, liver, kidney, gastrointestinal tract, etc.(Arti et. al., 2014). Nanoparticles are defined as a solid particles or dispersion in which the drug particles are dissolved or mixed. Nanoparticles size ranges from 10-1000 nm. They mainly work on site specific drug delivery system. The drug is enclosed in a polymer matrix. Nanoparticles may or may not be biodegradable. The nanotechnology in the form of medicine distributes the medicines in a proper and balanced way and spreads the drug rapidly. There are various methods of preparation, the nanoparticles, nanospheres and nanocapsules are obtained. Nanoparticles are mainly formed by (Mishra et al., 2010)

1) Preformed polymers dispersion
2) Monomers polymerization
3) Ionic gelation / Hydrophilic polymers coacervation.

Requirement of nanoparticles: (Mishra et al., 2010)

They are pharmacologically active substances which act on the site specific drug delivery system act as sustained drug and have controlled release properties. It mainly increases the stability and bioavailability of the drug.

Advantages of Nanoparticles: (Saba et al., 2010)

1. Controlled release drug delivery
2. Due to their small size the particles can easily penetrate the tissues and flow in the blood vessels.
3. Patient compliance
4. Increases the bioavailability of the drug.
5. Site specific drug delivery
6. Can be given through various drug delivery routes for example by parenteral, oral, nasal routes.

Limitations of Nanoparticles:

1. Due to their smaller size they have larger surface area, so they become more reactive in the cellular environment.
2. Sometimes occurs particle packing, making handling difficult.

Applications of Nanoparticles: (Saba et al., 2010)

1. Provides controlled drug delivery of poorly soluble drugs.
2. Less toxic and high stability.
3. Targeted drug delivery.
4. Systemic delivery of drugs which are water soluble.
5. Gene and drug delivery.

Herbal Formulation:

Herbal Formulation: In the ancient times the gold, silver, zinc, iron are converted into bhasma, tey are mainly in the form of powder. They are used as medicines mainly they are taken in the form of medicines. These forms of medicines are therapeutically active as compared to the tablet form. The particle size of the swarna, bhasma is 56 nm. (Kumari et al., 2012)Formulation of herbal medicine is an ancient procedure based on the Ayurveda system known as herb mineral preparations.Herbal medicines have a wide range of bioactivities due to their complex chemical structure; they show different active components and efficacy. Herb preparation and extraction procedure is the initial step for the chemical identification process of the active constituents.This step makes the downstream processes possible, including bioactive component identification, isolation and characterization (Kumar et al., 2013)

Herbal medicines in the nanoparticles improve various factors of the medicines that made the herbal drugs a powerful therapeutic solution to the organisms. The activity of herbal medicine mainly depends upon the active constituents present in them. The herbal medicines are mainly hydrophobic

in nature and poorly water soluble (Nishikant et al., 2012). These properties of the herbal medicines mainly affect the bioavailability of the drug. So in this case nanoparticles is used to increase the solubility of the herbal drug and site specific drug delivery to improve the various factors such as bioavailability, solubility of the drug, patient compliance. The nanoparticle enhances the therapeutic and pharmacokinetic index of the drug. It mainly enhances and improves the targeted delivery and combination therapy. So the herbal drugs have occupied a lead position in pharmacopoeias. (Vani et al., 2016)

PLANT PROFILE:

Figure: Fresh leaves of fenugreek **Figure: Seeds of fenugreek**

FIGURE: Leaves and Seeds of Fenugreek *(Trigonella foenum-graecum)*

Botanical Classification:

Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Fabales
Family	Fabaceae
Genus	Trigonella
Species	T. foenum-graceum
Synonyms	Methi,Greek Hay, Chandrika, Trigonella
Binomial Name	Trigonella foenum-graceum

Description:

Fenugreek is an annual herb. Seedlings grow in erect, semi erect or branched based. Its height is 30-60 cm. the leaves are compound, trifoliate, and white to yellow flowers and pointed like pods of 3-25 cm long. It is a self pollinating annual herb. The leaves, flower, ovary calyx, roots, corolla, stigma, style,pod seeds, anthers and stems are the major parts of the plant. An If the seeds are cut in

the transverse section, in which the hilum lies, it is found that larger lobe contains two accumbent cotyledons and the smaller contains the radical part. When it is soaked in water, the endosperm swells and yields mucilage to surrounding liquid (Nasroallah et al., 2013)

Distribution:

Trigonella foenum-graecum L. is an annual legume crop mainly grown for multiple uses in many parts of the world. Total 260 species are distributed throughout the world].The species of Trigonella have been found on the continents of Asia, Europe, Africa and Australia. Fenugreek was also cultivated in parts of Europe, northern Africa, west and south Asia, North and South America and Australia (Petropoulos et al., 2002)

Parts Used:

Whole plant leaves and seeds.

Phytochemical constituents:

The main chemical components of fenugreek are (Amin et al.,2005). The seeds mainly contain large percentage of folic acid, vitamins, saponins, amino acids, disogenin, gitogenin, neogitogenin, homorientin, neogigogenin, trigogenin (Kim su ji et al.,2006,). The young seeds contain carbohydrate and the endosperm of the seed contain galactomannan. Fenugreek seeds containalkaloids; also contain fiber, 4-hydroyisoleucine and fenugreekine, a component that may have hypoglycemic activity. Fenugreek contains mucilagin which provides the soothing and relaxing of inflamed tissues. Fenugreek seeds are anti-inflammatory, hypoglycemic, laxative, demulcent, deobstruent, emollient, uterine tonic, and useful in burning sensation (Moorthy et al.,1989)

Fenugreek influence on the diabetes:

Previous studies shows that the mechanism of action of fenugreek on animal revealed the ability of diatry fibersto delay gastric emptying, suppress release of the gastric inhibitory peptides and insulinotropic hormones (Srinivasan et al.,2005). Fenugreek seeds improve the diabetes and suppress the glycosuria.It contains different types of fibres which helps in control of diabetes. Fenugreek has an alkaloid “trigonelline”, which has an effect on glycosuria. the mechanism of action of fenugreek is to delay the gastric emptying , slow the carbohydrate absorption and inhibit the glucose transport. It improves the glucose utilization in peripheral tissues and increases the number of insulin receptors in Red blood cells and thus demonstrating potential anti-diabetec effects both on the pancreas and other sites. National Institute of Nutrition, India have demonstrated the beneficial effect of Fenugreek in both type-1 and type-2 diabetes with the help of different clinical trials on animals (Srinivasan et al., 2006).

Medicinal uses of fenugreek: (Nasroallah et al., 2013)

1) Treats diabetes and reduce cholesterol: it is used to reduce the blood glucose level in the blood.

2) Improves digestion: it flushes out the harmful toxins and also improves the memory too.
3) Prevent hair loss: fenugreeks are high source of proteins. It helps in treating baldness, thinning of hair and hair fall.
4) Antidote for skin problems
5) Fenugreek helps to attain the hormonal balance in women and helps in enlargement of breasts.
6) It Prevents Dandruff & Strengthens the Hair. Helps to prevent hair loss also.
7) It reduces the appetite , relieves fever , alleviates swelling and reduces body fats.

AIM:

To prepare nano structured formulation of fenugreek oil and sequential establishment for the anti-diabetic potential of the formulation.

OBJECTIVE:

1. To prepare nano structured formulations.
2. To characterize the formulation and its development.
3. To evaluate the nano-structured formulation for its pharmacological assessment.
4. Development and sequential establishment for anti-diabetic potential of the formulation.
5. Screening of the medicinal herb extracts for their ant diabetic effect in the animal model.

REVIEW OF LITERATURE:

1. Kamakhya Kumar et al., 2015, concluded that 2DM is not always sufficient, mau require some additional approach. In the study, the evaluation of the efficacy of the fenugreek seeds for controlling the glycemia and dyslipidemia in patients with type 2 DM. Several studies were conducted to observe the efficacy of the fenugreek seeds for the treatment of diseases. Slight inhibition in the activity of gluconeogenic enzymes was also noticed, proving there¬by that the active compound acts at both pancreatic and extrapancreatic sites. Thus, the studies in humans confirm that crude fenugreek seeds including trigonelline and others do have a good overall effect on DM and its complications

2. Dharmaseelan sarasa et al., 2012 concluded that. The compound present in the fenugreek have caused regeneration of beta cells effecting normal secretion of insulin, they concluded that it might be possible that the extracted compound might cause regeneration of beta cell. The liver glucose shows a increase with blood glucose level. It is possible that the compounds present in the extract might have caused. In the 1st day experimental animals the blood glucose level dropped to 110.12 ± 0.03 mg / 100 ml and there was a gradual reduction in the 3rd day and 5th day experimental animals. A near normal value of 96.72 ± 0.05 mg / 100 ml was observed in the 7th day experimental animals. It is evident the administration of TFSE has brought down the blood sugar level significantly. However, the administrations of TFSE alone did not show any particular change in blood glucose level of control animals.

3. Bharathi sambandam et al., 2016 concluded from the work that, solvent plays an important role in the extraction of plant constituents. quercetin has quercetin has been extracted from the leaves of Trigonella foenum-graecum.the identification of quercetin was done by FT-IR, NMR and mass spectroscopy. The extracted compound showed the increased antioxidant activity with an increase in the treated concentrations.

4. Manjeshwar shrinath baliga et al., 2017, concluded that several researches onmethi shows thatit possess anti-diabetic effects in alloxan and streptozotocin induced diabetic rats. The methi leaves and seeds are useful from ancient times but during these days several clinical trials are done to discover other therapeutic values of the medicinal herbs. They concluded that aqueous extract of leaf it possess a hypoglycemic effect in normoglycaemic and the oral route was safe and devoid of any systemictoxic effects

5. Mehedee hasan et al., 2016 concluded that both boiled and unboiled fenugreek is capable of reducing the blood sugar level. In the research boiled and liquid extract of fenugreek were given to the9 patients. The blood glucose level before administration were estimated, this step was repeated for 30 days. Their blood glucose level has been estimated in 15 days, 30 days,60 days

and 90 days interval. In Both cases fenugreek extract whether it would be boiled or unboiled reduces blood sugar. From some other study it has been seen that it requires minimum ten consequent days to have a physical effect of fenugreek in case of other dosage form like tablet,capsule,Crushed-seeds,powders etc. Almost all patients have shown a positive outlook and satisfactory approach to this particular dosage form of fenugreek,though some of them informed some unusual effect and side effects include:

6. Rita kiss et al., 2017 Oral preparation of fenugreek seeds improves glucose metabolism by its insulin-sensitizing effect, they conclude that it would to repeat these experiments on a number of patients who manifest T2DM. Our results presented here suggest that members of MCH-related signal transduction pathways could become new molecular targets of drug discovery and development in the future. So they concluded that those patients whose baseline GIR parameters was the lowest the fenugreek-associated increase in insulin-sensitivity was highest.

7. Walvekar M V et al., 2015 they concluded that after the treatment of fenugreek seed extract and fenugreek nanoparticles, the level of per oxidation and fluorescence product was decreased suggest that FSE and FNPs administration in diabetic mice reduce LPO and MDA product possibly by decreasing free radical formation and increasing antioxidant. The administration of fenugreek seed extract may ameliorate tissue dysfunction, antioxidants are known to improve tissue integrity. It was concludedthat fenugreek seeds are having antioxidant and anti per oxidative properties It concludes that,the fenugreek nanoparticles is best antidiabetic and antioxidant than fenugreek seed extract.

8. Promy virk et al., 2018 the phytochemical constituents of the seed extracts acts as a reducing agent and stabilizes the structure. The procedure of synthesis is simple, economic, nontoxic and efficient. The present synthesis approach could be extended as a potential nano therapeutic strategy in the management of type II diabetes and other metabolic disorders. The synthesis of Au/Ag nanocomposite with other phytocompounds could be agood approach in the field of nanomedicine. It is found that the possible reducing agents are flavonoids and the capping materials responsible for stabilization are the proteins present in the seed extract.

METHODOLOGY:

1. **Preparation of biomass:**

   For conducting the research work; firstly, the *Trigonellafoenum-graecum* will be collected and authentication of the plant will be done by some reliable company (Rajesh et al., 2015).

2. **Fenugreek seed extract:**
   The fenugreek seed will be mixed with distilled water and kept for 24 hours at 2-4^{o} C. then the filtrate will be centrifuged. The weight will be measured after the centrifugation and further will be used for the experiment.

3. **Chemicals:**
   Purchase of Suitable chemicals for the further experiment.

4. **Synthesis of nanoparticles**

5. **Characterization of prepared nanoparticles**

   Instrumental Analysis Scanning electron microscope analysis (SEMA) and Scanning electron microscope (SEM) images tests will be done for the analysis of size and shape of prepared nanoparticles.

6. **Screening of the herb for their antidiabetic effect in the animal model**

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